

EVALUATION OF THE OXIDATIVE STRESS STATUS AND HEPATIC DETOXIFICATION FUNCTION AFTER THE INTAKE OF FOODS CONTAINING HIGH ACRYLAMIDE LEVELS

EVALUAREA STRESULUI OXIDATIV ȘI A FUNCȚIEI DE DETOXIFIERE HEPATICĂ ULTERIOR INGESTIEI DE ALIMENTE CU UN CONȚINUT BOGAT ÎN ACRILAMIDĂ

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Abstract. *The present experiment evaluates the pathological changes in oxidative stress and hepatic detoxification function after the intake of foods containing high acrylamide levels, by the means of biochemical analysis (superoxide dismutase, catalase, free sulfhydryl groups). The experimental model included 4 groups of Wistar rats: reference group (standard food), control group (the drinking water was replaced by an acrylamide solution), FP group (fried potatoes and standard food), T group (toast and standard food). The results reveal that the intake of foods containing high acrylamide levels leads to the following modifications: decrease of CAT for control group, FP group, and T group; decrease of SOD for control group, FP group, and T group; decrease of free sulfhydryl groups for control group, FP group, and T group. In conclusion, the intake of foods with high acrylamide levels has an important negative impact upon the oxidative stress status and hepatic detoxification function.*

Key words: acrylamide, fried potatoes, toast, oxidative stress, hepatic detoxification function.

Rezumat. *Prezentul experiment evaluează modificările patologice ale stresului oxidativ și funcției de detoxifiere hepatică, prin determinarea biochimică a superoxid dismutazei, catalazei și grupărilor sulfhidril libere. Modelul experimental a inclus 4 loturi de șobolani Wistar: lotul de referință (hrană standard), lotul de control (apa de băut a fost înlocuită cu soluție de acrilamidă), lotul FP (cartofi prăjiți și hrană standard), lotul T (hrană standard și pâine prăjită). Rezultatele analizelor biochimice relevă faptul că ingestia alimentelor cu un conținut bogat în acrilamidă determină: scăderea activității CAT pentru lotul de control, lotul FP și lotul T; scăderea activității SOD pentru lotul de control, lotul FP și lotul T; scăderea concentrației grupărilor sulfhidril libere pentru lotul de control, lotul FP și lotul T. În concluzie, ingestia alimentelor cu un conținut bogat în acrilamidă are un important impact negativ asupra statusului stresului oxidativ și a funcției de detoxifiere hepatică.*

Cuvinte cheie: acrilamidă, cartofi prăjiți, pâine prăjită, stres oxidativ, funcție de detoxifiere hepatică.

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INTRODUCTION

The preferences of human beings for a better taste, colour and texture of every day food persuaded them to transform cooking into culinary art, by developing new food processing technologies, that allowed the formation of different chemical compounds that improve the organoleptic qualities of foods. On the other hand, the rapid rhythm of this century's living determined man to prefer semi-prepared foods, which implied the use of different food additives, many of them with harmful effects upon health. In both cases, the living food and its benefits have been neglected, unfortunately with undesirable effects upon human health.

Acrylamide, a process-induced food toxicant with an alarming toxicological profile, was found in fried, roasted, baked and grilled foods by the Swedish researchers in April 2002 (Eriksson, 2005; Friedman, 2003). During the first years after its discovery, acrylamide received considerable attention, due to the significant exposure of consumers to foods containing high acrylamide levels. The toxicity of acrylamide has been extensively investigated, indicating its neurotoxic and genotoxic potentials, its reproductive and developmental toxicity, and also the carcinogenic potential in rodents. Acrylamide has also been classified as a "probable human carcinogen" (Burlacu et al., 2008; Dybing et Sanner, 2003). The „acrylamide" subject has lately been abandoned, due to the lack of evidence of the epidemiological studies.

The present experiment evaluates the pathological changes in the oxidative stress status and the hepatic detoxification function after the intake of foods containing high acrylamide levels (fried potatoes and toast), by the means of biochemical analysis (superoxide dismutase, catalase, free sulfhydryl groups).

MATERIAL AND METHOD

Analysis instruments

The biochemical analysis was achieved with an open system EOS 880 PLUS semi-automatic analyzer.

Experimental model

All the experimental proceedings in this experiment were achieved according to the international ethic regulations and were approved by the Ethics Commission of the University of Medicine and Pharmacy "Gr. T. Popa" Iași.

A total number of 24 male rats, Wistar strain, having body weights comprised between 180 and 220 g, were divided into 4 groups, as follows:

- reference group - fed with standard food and drinking water;
- control group - received the same diet as the reference group, except for the drinking water, which was replaced by an acrylamide solution 0.1 µg/L (the maximum allowed limit regarding the presence of acrylamide in the drinking water, according to the European Union legislation);
- FP group - the food supply was made up of French fries (given in the morning, around 8.00 o'clock), and the reference group's diet (at 14.00 o'clock);
- T group – the diet consisted of sliced white bread, toasted for 3 minutes into the electric toaster (8.00 o'clock) and the reference group's diet (at 14.00 o'clock).

The animals were housed in separate cages, in order to control their diets. The experiment was conducted over a period of 90 days.

Biochemical investigation

At the end of the experiment the animals were anesthetized with ketamine i.p. (75mg/kg) and blood samples were collected by the puncture of the cord with a Vacuette ® system and submitted to biochemical analysis, which aimed to evaluate the oxidative stress status (by determining superoxide dismutase - SOD, catalase – CAT) and the hepatic detoxification function (by determining free sulfhydryl groups).

Statistical interpretation

Statistical data were processed by program StatsDirect version 2.7.2 (2008). The accepted significance threshold is 95%, i.e. $p < 0.05$. The value of p is inversely proportional to the statistical significance. Statistical interpretation of data considered the differences corresponding to a significant threshold as follows: $p > 0.05$ statistically insignificant; $p < 0.05$ statistically significant; $p < 0.01$ highly statistically significant; $p < 0.001$ very highly statistically significant.

RESULTS AND DISCUSSIONS

Evaluation of the oxidative stress

a. Determination of serum catalase

The first parameter indicating the presence of reactive oxygen species with peroxide structure is catalase, enzyme with mitochondrial and peroxisome localization. The analysis of the activity for this enzyme, indicates the involvement of oxygen free radicals in the annihilation of this enzyme in the serum of the animals exposed to the chronic intake of acrylamide (fig. 1). If for the reference group the catalase activity amounts to 531.625 ± 46.771 U/L, values decrease to 496.2 ± 10.799 U/L for the control group, highlighting the interference of the enzyme in the inhibition of free radicals (fig.1). Catalase activity returns to levels close to those of the reference group for the animals exposed to chronic intake of acrylamide through consumption of toast (509.24 ± 8.549 U/L), while the lowest enzyme activity is recorded for the animals fed with fried potatoes (495.64 ± 14.931 U/L) (fig. 1).

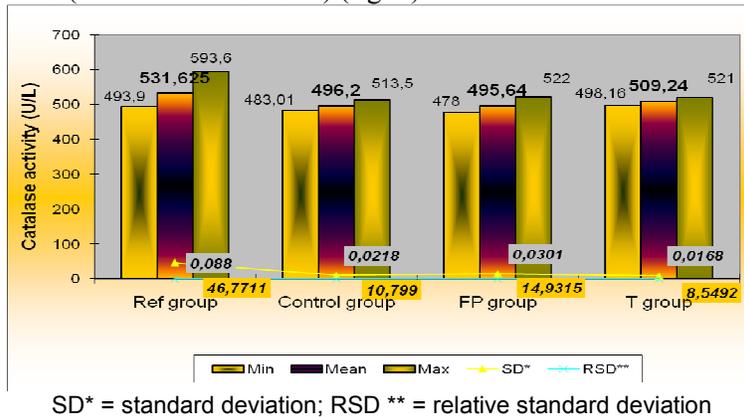


Fig. 1 - The activity of catalase

b. Determination of superoxide dismutase

The second parameter of oxidative stress is superoxide dismutase, metalloenzyme discovered by Irwin Fridovich, which uses as substrate the superoxide radical (Temneanu *et al.*, 2011). The variations of this enzyme reveal its implications in the eradication of free radicals for all experimental groups (fig. 2). Therefore, if for the reference group the SOD activity amounts to 415.63 U/mL \pm 4.9979, for the control group (the group exposed to chronic administration of the acrylamide aqueous solution) the value significantly decreases to the amount of 388.61 U/mL \pm 19.617, demonstrating the intervention in the annihilation of superoxide radicals (fig. 2).

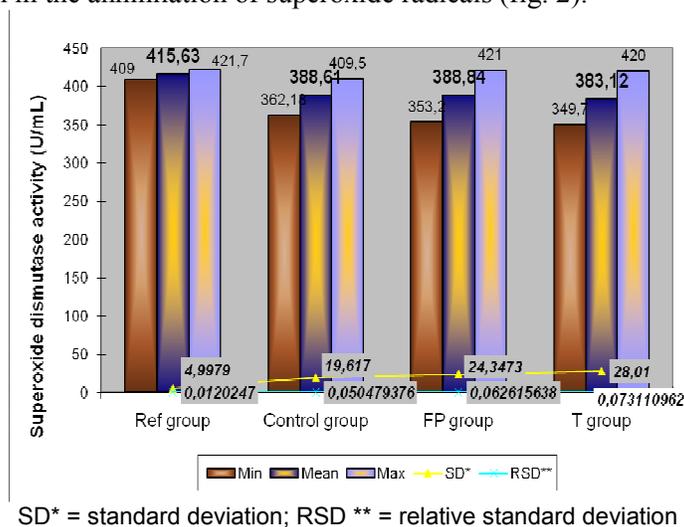


Fig. 2 - The activity of superoxide dismutase

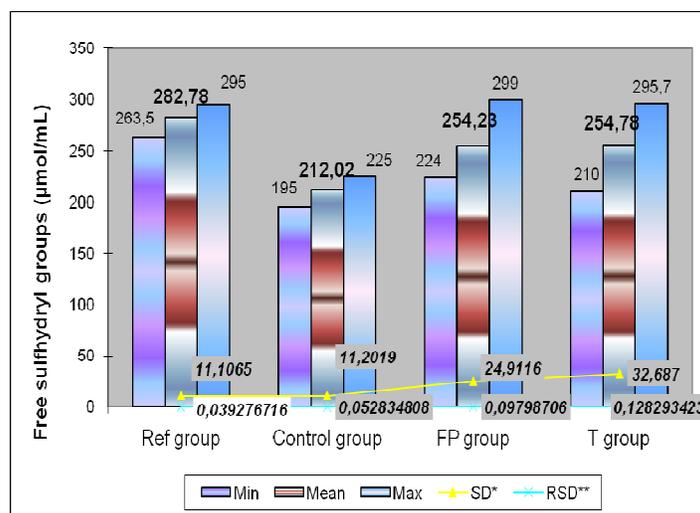
Evaluation of the liver detoxifying function

c. Determination of free sulfhydryl groups

Glutathione (γ -glutamyl-cysteinyl-glycine) is a nucleophilic tripeptide, which has a critical role both in combating reactive oxygen species, but also in the process of detoxification, removing harmful metabolites after conjugation reactions, reactions conducted under guardianship enzyme glutathione-S transferase (Coleman *et al.*, 1997).

Results of the quantitative determination of free sulfhydryl groups in the serum of the laboratory animals are given in fig. 3. The analysis of the obtained values supports the antioxidant and detoxifying roles of glutathione. If for the reference group, the concentration of free sulfhydryl groups has a value of 282.78 \pm 11.106 μ mol/mL, the control group reflects a very significant reduction of 70 units. This negative variation shows either the intervention of glutathione in combating overload cellular free radicals of oxygen, or the involvement in the mechanisms of metabolism and conjugation of toxic amide. For the animals of the

groups fed with fries and toast an uniform increase compared to the control group values can be seen, the values still being below those of the reference group ($254.23 \pm 24.911 \mu\text{mol/mL}$ for the animals fed with fried potatoes and $254.78 \pm 32.687 \mu\text{mol/mL}$ for the animals fed with toasted bread) (fig.3).



SD* = standard deviation; RSD ** = relative standard deviation

Fig. 3 - The activity of free sulfhydryl groups

CONCLUSIONS

1. The variations of the catalase activity emphasize its involvement in inhibiting the reactive oxygen species by reducing its concentrations in the serum of the groups exposed to the chronic acrylamide intake, either by the 0.1 g/L acrylamide solution or by fried potatoes and toast.

2. The second parameter relevant for the oxidative stress, superoxide dismutase, reveals statistically significant modifications of its serum levels (up to 27-30 units) that demonstrate its involvement in the eradication of the oxidative stress.

3. The analysis of the free sulfhydryl groups sustains the double role of glutathione (antioxidant and detoxifying agent) by the significant decrease of its serum values, mainly for the group exposed to the acrylamide aqueous solution.

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